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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/897,988	07/05/2001	Yuta Nakai	210669US0	1677

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EXAMINER

MARVICH, MARIA

ART UNIT PAPER NUMBER

1636

DATE MAILED: 04/09/2003

124

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

09/897,988

Applicant(s)

NAKAI ET AL.

Examiner

Maria B Marvich, PhD

Art Unit

1636

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 09 January 2003.
- 2a) ☐ This action is FINAL. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1-9 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1-9 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☒ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on 09 January 2003 is/are: a) ☐ accepted or b) ☒ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.
If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

- 13) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- * See the attached detailed Office action for a list of the certified copies not received.
- 14) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____.
- 4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: _____.

Art Unit: 1636

DETAILED ACTION

This action is in response to an amendment filed 1/9/03. Claims 1-9 have been amended. Claims 1-9 are pending in this application.

Response to Amendment

Substitute formal drawings have been submitted which fail to comply with 37 CFR 1.84. Please see the enclosed form PTO-948.

The substitute specification has been entered.

The rejection of claims 1-5 and 7-8 are withdrawn in light of applicant's comments. Specifically, applicant has pointed out that the substance produced in Ciccognani et al. is not secreted and therefore differs from the instant invention in which the target substance to be produced is secreted and collected from the medium.

The rejection of claim 7 under 112, second paragraph, has been withdrawn in light of amendment to the claim.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of producing a target substance utilizing

Art Unit: 1636

E. coli with an enhanced SoxM type oxidase or NDH-I activity and deficient cytochrome bo type oxidase or NDHII activity, it does not provide an enabling disclosure for said method using any microorganism with any enzyme constituting the respiratory chain pathway with high energy that is enhanced and/or with low energy efficiency that is deficient. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and *In re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

1) Unpredictability of the art. The instant application claims a method for the production of target substances following strain improvement. These improved microorganisms are engineered to contain altered genes of the respiratory chain pathway in which enzymes of high efficiency are enhanced by amplification of native genes, mutagenesis of native promoters or the introduction of regulatory genes. Alternatively or in concert with the aforementioned mutation, enzymes of low efficiency of the respiratory pathway are made deficient by mutation or deletion. The strain is then used for the production of any target substance.

As Bailey (Science, June 1991) teaches, metabolic engineering “enables construction of metabolic configurations with novel and often beneficial characteristics” but “at present, metabolic engineering is more a collection of examples than a codified science” (page 1668, column 1, paragraph 2 and 3). Furthermore, he teaches “Many studies have shown the feasibility of metabolic engineering methods without achieving the yields, rates or titers (final concentrations) required for practical processes.” (page 1668, column 1, paragraph 3). The obstacles Bailey details are that the cell has complex cellular responses to genetic perturbations that complicate predictive design. The complex cellular responses include an inability to anticipate further reactions which leads to iterative cycles of genetic modifications, an inability to predict metabolic consequences following the transfer of heterologous genes into the cell as well as rearrangements and deletions of chromosomal and plasmid DNA.

More recently, Parekh et al. (Appl Microbiol Biotechnol., 2000) teach that “...heterologous protein expression in bacterial or fungal systems offers a significantly complex ,metabolic network. The rate-limiting enzymatic reactions or flux nodes are unknown in most if not all pathways and probably change with each new culture. Thus with limited knowledge of the physiology and genetics associated with the production of each molecule of interest one is often led to an empirical approach to strain improvement. (page 288, column 1, paragraph 1).” Further problems are encountered with the need to scale-up processes such as false positives are encountered and an inability to maintain the same physical environment (page 299, column 1, paragraph 2-3).

Specifically, for the system designed in the instant application, the art of discernment of energetic efficiency is a contested topic. Identifying genes other than

Art Unit: 1636

NDH-I, NDH-II, cytochrome bd and cytochrome bo is not guaranteed as the means to determine the efficiency of energy of the proteins these genes encode is an unpredictable art. As taught by Neijssel and de Mattos (Molecular Microbiology (1994) 13(2), 179-182), "the efficiency of energy conservation by bacterial respiratory chains using intact cells could not (and still cannot) be determined as simply as in mitochondria and cell-free preparations yielded unreasonably low P/O ratios" (page 180, column 1, paragraph 1). They further teach "it can be shown that there are many energy-spilling reactions in the cell whose physiological functions are sometimes unclear (e.g. futile cycles), but whose activity in vivo may well vary with growth rate. It is extremely difficult if not impossible to determine the activities of these reactions in growing cultures, and this implies that one cannot derive a reliable estimate of the efficiency of the respiratory chain from measurements of growth yields" page 180, column 2, paragraph 2).

2) State of the art. The state of the art at the time of invention included knowledge of respiration systems in *E.coli* rooted in many years of analysis. The analysis of energetic efficiency and the detailed analysis of respiratory pathways are still under study. We are taught in the specification that the enzymes cytochrome bd type oxidase and NDH-II are low efficiency enzymes while cytochrome bo type oxidase, SOX M, bcl complex and NDH-I are high efficiency enzymes. The prior art does not identify any other genes for which the state of their energy efficiency has been determined.

3) Number of working examples. The specification provides by way of example of claimed invention developing a system with amplified bo type oxides (cyo operon) or deletion of the gene encoding NDH-II. Several means of amplification are provided including gene amplification, promoter mutations. Homologous recombination and

Art Unit: 1636

mutation of *ndhII* are suggested to create a deficiency in this enzyme. Overproducing cyo strains and deficient NDHII *E. coli* strains are exemplified. The effect of these strains in the production of L-lysine, l-phenylalanine and L-threonine is also provided as to strains, growth conditions, and assays for production.

4) Amount of guidance provided by applicants. Guidance has been provided for the generation of *E. coli* with specific alterations in the expression levels of bo and bd type oxidases as well as NDHI and NDHII strains. This includes guidance as to the means of over expression through gene amplification, promoter enhancement and mutagenesis of the bo type oxidase. Reduction or elimination of NDH-II is suggested through mutation or recombination. Examples of microorganisms are those in which SoxM or NDH-1 are enhanced and cytochrome bd or NDH-II are reduced. The guidance provided by the applicants does not provide for how to predict what other strains of bacteria with altered high and low efficiency enzymes would be suitable.

5) Nature of invention. The invention recites a method for producing target substances through genetic manipulation of bacterial strains. It relies primarily on the fields of microbiology and recombinant technology.

6) Level of skill in the art. The level of skill in the art covering this invention was high at the time of invention. However, given the unpredictability of the art, the poorly developed state of the art and the lack of guidance presented by applicant, the skilled artisan would have needed to conduct undue and excessive experimentation to practice the claimed invention.

Art Unit: 1636

7) Scope of the invention. This invention is broad in scope in that it recites a method to produce any target substance through genetic manipulation of any enzyme in the respiratory pathway

Given the above analysis of the factors which the courts have determined are critical in determining whether a claimed invention is enabled, it must be concluded that the skilled artisan would have had to have conducted undue experimentation and excessive experimentation in order to practice the claimed invention.

Claims 1-9 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants claim a genus of microorganisms constructed from a parent strain that has a respiratory pathway of high energy efficiency and a respiratory pathway of low energy efficiency and that is a mutant or genetic recombinant having either or both an enhanced respiratory chain pathway of high energy efficiency and a deficient respiratory chain pathway of low energy efficiency.

The written description requirement for genus claims may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with known or disclosed correlations between function and structure, or by a

Art Unit: 1636

combination of such characteristics sufficient to show that the applicant was in possession of the claimed genus. The invention uses as an essential element for the production of target substance, improved microorganisms derived from parent strains that are engineered to contain altered high and low energy efficiency genes of the respiratory chain pathway. The structure of a species is disclosed as the specification discloses that the *E. coli* strains W3110 and VKPM B-3996 are parental strains for the construction of the overproducing cyo strains and deficient NDHII strains. The specification does not disclose relevant identifying characteristics of the nature of the mutation or genetic engineering sufficient to describe the steps to attain the mutant strains with the derived high energy or low energy efficiencies in such full, clear, concise, and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention. Nor does the specification disclose a correlation between the mutations or genetic changes in the exemplified cell, *E. coli*, and those required in other organisms to produce the necessary changes in the respiratory pathway for use in the claimed invention. For inventions in an unpredictable art, adequate written description of a genus cannot usually be achieved by disclosing only one species within the genus. Furthermore, the specification and the prior art has not established a strong correlation between the structure of mutated or genetically altered enzymes and their function in altering respiratory pathways in the organism and one skilled in the art cannot predict with a reasonable degree of confidence the structure of the claimed invention from the recitation of its function. Therefore, the skilled artisan cannot conclude that the applicant was in possession of the claimed invention.

Art Unit: 1636

Response to Arguments

Applicant traverses the rejection of claims 1-9 under 112, first paragraph, on pages 5-8 of the amendment filed 1/9/03. Applicant argues that the Bailey reference is old and therefore, a newer reference is required to argue that the claims are not enabled. As well, applicant was unable to address the Neijssel reference, as it was not included in the office action. Finally, applicant argues that the applicant has exemplified the use of enzymes other than the enzymes used in the present Examples and the same results as those present in the Example of the present application may be obtained and without undue experimentation.

The arguments filed 1/9/03 have been considered and in response the rejection of claims 1-9 under 112, first paragraph have been resubmitted with additional references under anon-final office action. The specification, as well as the prior art, solely teaches that the cytochrome bd oxidase and NDH-II are low efficiency enzymes while cytochrome bo type oxidase and NDH-I are high efficiency enzymes. No other enzymes are exemplified. Neither the prior art nor the specification indicates that the means to identify any other respiratory enzyme with low or high efficiency is available. It is not clear that applicant's invention can be performed with any microorganism with any enzyme constituting the respiratory chain pathway with high energy that is enhanced and/or low energy efficiency that is deficient.

No claims are allowed.

Art Unit: 1636

Conclusion

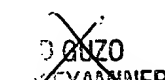
Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B Marvich, PhD whose telephone number is (703) 605-1207. The examiner can normally be reached on M-F (6:30-3:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel, PhD can be reached on (703) 305-1998. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 305-4242 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Maria B Marvich, PhD
Examiner
Art Unit 1636

April 7, 2003


DAVID GUZO
PRIMARY EXAMINER


DAVID GUZO
EXAMINER